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A

FURTHER CONTRIBUTION

TO THE

NATURAL HISTORY OF BACTERIA,

AND THE

GERM THEORY OF FERMENTATIVE CHANGES.

BY

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1873.

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(*With Plates XIX, XX, XXI.*)

In April of this year I communicated to the Royal Society of Edinburgh some of the results of a protracted investigation into various circumstances connected with the appearance and growth of minute organisms in fermentable substances.[1] During the time that has since elapsed I have continued to prosecute the enquiry, and have obtained various new and striking confirmatory facts, a selection from which will form the subject of the present paper.

In the former communication observations were related which led me to conclude that in some minute species of hyphomycetous fungi, the spores (conidia) produced upon their filamentous branches germinate in three distinct ways; first, they may form comparatively thick sprouts, which become young plants, like the parent; second, they may multiply by pullulation like the yeast plant, and under some circumstances this toruloid growth[2] may continue for an indefinite period, though the resulting progeny will, under favouring conditions, reproduce a fungus like the original; and, thirdly, the conidia may shoot out sprouts of exquisite delicacy which break up into bacteria. In accordance with

[1] For an abstract of the chief points of this communication "On the Germ Theory of Putrefaction and other Fermentative Changes," see 'Nature,' July 10th and 17th. It is in course of preparation for publication *in extenso*, in the 'Transactions of the Royal Society of Edinburgh.

[2] Considering the differences among authors in the use of the term torula, it seems justifiable for the sake of convenience to retain the old sense, as applicable to organisms like the yeast plant.

this mode of origin of bacteria it was shown that such organisms, like the fungi from which they are derived, are of various totally distinct kinds, manifesting their differences both morphologically and still more physiologically by the characters of the fermentative changes to which they give rise, and by the circumstance that some sorts refuse to grow at all in media in which others thrive. Some of the species exhibited most remarkable variations in size, form, and movement when introduced into different media, and sometimes gave indications of their fungoid origin by indubitable branching, and, in the thicker forms, by the presence of nuclei or vacuoles. Yet, however much any such modification might differ from the form in which the species was seen in another medium, the latter variety could be reproduced at pleasure by reintroduction into the habitat in which it was originally seen.

Hence any classification of bacteria hitherto made, from that of Ehrenberg to that of Cohn,[1] based upon absolute morphological characters, is entirely untrustworthy. In order to determine the species of any particular specimen it is necessary to take into account not merely its appearance, but also the character of the medium in which it occurs. Even then mere morphology will often entirely fail us unless we are able to ascertain the physiological characters. And even these appear by no means constant; for we shall in the present paper see reason to believe that one and the same bacterium may differ at different times in its fermentative effects on one and the same organic solution.

It is obvious that to trace the modifications of any one such organism through a series of successive habitats would be an utter impossibility if bacteria or any kind of fungi were liable to be evolved from the mere chemical constituents of the liquids employed; and thus the investigation, though not undertaken for the purpose of combating the doctrine of spontaneous generation, has afforded the strongest possible evidence against it, and in favour of the germ theory of fermentative changes. For even in organic liquids such as milk, in which spontaneous generation has been said to be most liable to occur, it required only a rigorous attention to experimental

[1] It is, however, only just to Prof. Cohn to state that he dwells largely upon the different physiological effects of different supposed species of bacteria, and sometimes makes them a ground of classification, more especially in the group of "pigment bacteria," which he distinguishes from others on account of the remarkable colouring matters to which they give rise. Nevertheless he relies in the main on absolute morphological characters. See "Untersuchungen über Bacterien," von Dr. Ferdinand Cohn. Beiträge zur Biologie der Pflanzen,' Zweites Heft, Breslau, 1872.

details to ensure the complete absence of either organic development or fermentative change, except where organisms were intentionally introduced. But when this was done, the particular species used for inoculation grew unmixed with others, attended by the chemical alterations characteristic of it.

In order to enable the reader to give credence to my statements, it is essential that I should describe in detail the mode of procedure in its most improved form. Let us take, as an example, the case of boiled milk. The first thing to be done is to ensure that the interior of the vessel in which the liquid is to be heated shall be free from any living organisms. This is done by subjecting a Florence flask to a very high temperature, after providing that the air which enters on cooling shall be effectually filtered of living dust by passing through asbestos which, I find, answers this purpose quite satisfactorily. The asbestos is placed, in a mass about a quarter of an inch thick, between two layers of tin foil sufficiently broad and long for wrapping round the junction of the neck of the flask and a glass cap that covers its mouth; and when it has been so arranged, fine iron wire is tied tightly round, so as to compress it firmly as well as retain it in position after the outer layer of tinfoil has been dissipated by fusion and oxidation. The flask, previously mounted in strong wire for convenience of holding with forceps and for suspension, is then roasted thoroughly over a large Bunsen's burner and hung up by its wire mounting to cool.

The next point is to introduce the milk without contaminating any part of the flask except the lower portion that receives the liquid. For this purpose a funnel is used sufficiently long to reach from some distance above the mouth of the flask to its bottom; and the exterior of the tubular part of the funnel is freed from living organisms by wiping it with a cloth, soaked in a strong watery solution of carbolic acid (one part of the acid to twenty of water), and drying it with a carbolized rag prepared by immersing it in a solution of one part of the acid in a hundred parts of anhydrous sulphuric ether and allowing the ether to evaporate.

This is much more convenient than heating the thick glass of the funnel, as I did in my earlier experiments; and I may add that throughout this investigation I have found great advantage from thus substituting the use of chemical antiseptic means for the employment of a high temperature when the former happens to be more convenient. And I may remark incidentally that the results have afforded most conclusive evidence of the efficiency of a strong watery

solution of carbolic acid for destroying minute organisms; for throughout the whole course of the experiments I have found cleansing with such a lotion exactly on a par in this respect with exposure to the gas flame.

The tube of the funnel, thus freed externally from living germs, is passed down to the bottom of the flask, the asbestos having been previously removed and the glass cap lifted after wiping its margin with carbolic lotion for the chance of any organism having been applied to it in the process, and a piece of carbolized rag being wrapped round the mouth of the flask and the funnel to exclude living dust, the milk is poured in so as to fill not more than the lower half of the body of the flask. The funnel is then withdrawn through the rag, scrupulous care being taken that its extremity, now contaminated with the milk, does not touch the side of the flask. A substantial piece of cotton-wool carbolized in the manner above described is then tied over the mouth of the flask to filter any air that may regurgitate during the next stage of the process, the heating of the milk. This is done by immersing the body of the flask in a saucepan of boiling water and retaining it there for about an hour, care being taken that the boiling water never sinks below the level of the milk. By this means we are sure that the milk has been throughout exposed to the temperature of 212° F. for the period desired, while the earlier parts of the process give us equal assurance that the whole interior of the flask above the milk is free from living organisms. The immersion of the flask in a bath of boiling water, for which I am indebted to a suggestion of Mr. Godlee, of University College, London, has three advantages over boiling by direct flame; it avoids frothing, which in the case of milk is extremely troublesome, and also the sputtering to which Dr. Roberts, of Manchester, has drawn attention;[1] it prevents any loss of water by evaporation, and so disposes of the vexatious question of whether the specific gravity of the liquid has not been so raised as to render it unfavorable for organic development; and lastly, it avoids any "burning" of the milk with its accompanying chemical changes.

The milk having been allowed to cool completely, a portion of it is decanted off into experimental glasses. These are plain "liqueur glasses," each provided with a glass cap shaped like a small evaporating dish (made to order at any glass work) and covered with a small glass shade standing on a square piece of plate glass. The glass plate has the double advantage of allowing the glass to be removed without disturbing

[1] See 'Nature,' Feb. 20th, 1873.

the glass shade, and also of preventing the air beneath the shade from acquiring an accidental odour such as is derived from wood or other porous substances, and interferes with judging of chemical changes by the sense of smell.

The glass shade and glass cap have in combination the effect of perfectly excluding all living dust, although, as neither cap nor shade is made to fit closely, a constant free interchange by diffusion between the air in the liqueur glass and the gases of the atmosphere is permitted. Hence, provided always that the liqueur glass and its cap are free from living organisms to begin with, and that the contained liquid is similarly circumstanced, the latter will remain for an indefinite period unchanged except by gradual loss from evaporation, till at length in the course of months it dries up into a solid mass.

Further I have found as a matter of experience that if the glass shade and cap are raised, in a part of the room free from draughts, for the purpose of inoculation of the liquid or withdrawal of a small quantity for examination, there is practically no risk of the accidental introduction of organisms, provided of course that the operations be nimbly executed and that any piece of apparatus introduced into the glass have been suitably purified. For it appears that organic germs are not nearly so abundant in the atmosphere as is sometimes assumed, and only a very small fraction of the portions of dust with which the air of an occupied room is loaded have such germs adhering to them. Thus, in one instance the sole result of exposure of a glass of uncontaminated urine for half-an-hour in my study was three plants of three different kinds of filamentous fungi, each growing from one point and enlarging thence in all directions, while the liquid remained otherwise unchanged in aspect, a fact which may probably be stated equally truly by saying that, of all the many particles of dust that fell in during that period, only three at most contained a germ capable of growing in urine.

Hence while it is most true that scrupulous care must be taken in these experiments, and that forgetfulness or slovenliness in their execution would be absolutely fatal to success, yet it is equally true that by the very simple means which I am now describing the observations may be made with a facility and precision that leave nothing to be desired.

The glass plate and shade are simply washed and dried with a towel, but the liqueur glass and its cap must be purified by heat like the flask. This is very simply done by bringing both to a high temperature over two spirit lamps or

Bunsen's burners, the liqueur glass being held in the hand by its foot and the cap in a pair of forceps; and the cap having then been placed on the glass, a substantial piece of cotton-wool with a bit of muslin beneath it (neither carbolized) is placed on the cap and tied firmly with fine iron wire round the glass beneath. The heat of the glasses ensures the destruction of organisms in the lower part of the cotton, which acts as a perfect filter during cooling; and though the muslin may be browned by the high temperature, no empyreumatic odour is occasioned in the glass nor any deposit on its sides.[1] The glasses having cooled, the wire ligature is cut and the cotton carefully removed, the muslin beneath serving the purpose of clearing off all portions of cotton at once, and the glass and cap are immediately placed on the glass plate beneath the shade.

A series of experimental glasses, say a dozen, having been thus prepared, it remains to charge them by decanting from the flask, and this is a matter which, at the risk of appearing tedious, I am compelled to describe in minute detail.

The process is effected by means of a syphon of glass tube with a calibre of about one eighth of an inch, the shorter leg rather longer than the height of the flask, and the other leg four or five inches longer. I find the most convenient way of purifying the syphon is to boil it, and in order to adapt it for packing into a saucepan, the glass tube is interrupted at intervals of about four inches with pieces of caoutchouc tubing, the shorter leg having one such india rubber hinge, and the longer leg two. They are tied firmly on the tube with fine wire, silver wire being used for the shorter leg, where iron might rust. In the longer leg one of the caoutchouc junctions serves the further important purpose of enabling the assistant to control the flow through the syphon by compressing the india rubber between the finger and thumb. A fourth piece of caoutchouc tubing is applied, without tying, to the end of the longer leg, for adapting a syringe. The syphon thus constructed is filled with water and boiled for half an hour, and while it is still in the hot water, one of the caoutchouc junctions of the longer leg is seized with catch forceps (previously heated) to prevent the syphon from emptying itself when taken out. The longer leg being now raised from the saucepan by aid of another pair of heated forceps, the syringe, which has been washed out with carbolic lotion, and the nozzle passed through the flame, is applied to

[1] It is only in my more recent experiments that I have thus employed the cotton, but there can be no doubt that while it scarcely adds to the trouble of the process, it must materially increase its security.

the terminal caoutchouc adapter. The shorter leg is next raised, and at once slipped through a hole in the middle of a piece of carbolized cotton wool, and then into the flask (whose cotton cap has been previously loosened, so as to be ready for removal), and the end of the leg being kept a little above the level of the liquid, to avoid mingling of the water in the syphon with it, the cotton is tied round the neck of the flask and the syphon. Then, as the syphon is intended to be left permanently adapted to the flask to serve for future decantings, it is needful to provide against the access of organisms to the moisture between the india rubber junctions of the longer leg and the glass tube. For this purpose, the catch forceps being removed, carbolized cotton wool is wrapped round each junction, and a piece of rag over this to enable it to resist wear, and tied securely round the glass tube above and below the caoutchouc. The syphon is now emptied of its water by means of the syringe, and the shorter leg being pushed down till its extremity is in the liquid, the syringe is again brought into operation till the syphon is seen to be full of milk. The assistant then compresses one of the caoutchouc junctions through its cotton investment, to prevent the milk flowing out when the syringe and its adapter are removed. This is done with fingers dipped in the carbolic lotion, and the apparatus is completed by slipping upon the glass tube that now terminates the syphon a circular piece of thin caoutchouc, about two inches in diameter, with a hole in the centre just large enough to admit the tube, so that it remains in position without further fixing. This caoutchouc plate is to serve as a screen to keep dust out of the glasses while they are being filled. To keep it level it is strengthened by a fine wire run through and through near its margin, and, to ensure freedom from living organisms, it is steeped for half an hour or so in the strong carbolic lotion; after which, as caoutchouc has the property of imbibing carbolic acid into its substance, the screen when dried retains a sufficient quantity of it to ensure the destruction of organisms that may come in contact with it. The experimental glasses, which as yet are covered with their shades at as short a distance as possible from the syphon, are successively exposed and charged, each being brought close to the syphon before the glass cap is raised, and then at once placed with its margin in contact with the caoutchouc screen, while the end of the syphon extends into the glass. The assistant is now directed to relax his hold upon the caoutchouc junction above, when the milk at once flows into the glass, and when this is about two-thirds filled, the flow is

again arrested by the assistant, the glass removed, the cap, held in the other hand of the operator, is reapplied, and the glass placed again under cover of the shade.

All the glasses having been charged, the caoutchouc screen is slipped off and a piece of carbolized cotton tied over the end of the syphon, which being raised to a higher level than the fluid in the flask the assistant finally relaxes his hold and the syphon empties itself into the flask, becoming occupied by air filtered by the cotton tied over the extremity. When at any future time another set of glasses are to be charged, all that is needful is to remove the cotton wool from the end of the syphon, readapt the syringe by means of a caoutchouc adapter, steeped for a short time in carbolic lotion, and then proceed as before. In this way we avoid the great loss of time involved in providing a fresh syphon for every fresh decanting, as I did in the earlier experiments.

The other experimental fluids employed in the observations about to be related were Pasteur's solution, turnip infusion, an "artificial milk," consisting of a solution of sugar of milk and white of egg in water, and urine.

In preparing the Pasteur's solution for this set of experiments I deviated from Pasteur's formula in two respects; viz. the proportion of the water and the source of the mineral salts. I doubled the quantity of water, so as to make the liquid, as I hoped, more favorable for the growth of some organisms, more especially after loss by protracted evaporation as occurs in my experiments, and tap water was employed instead of distilled, so as to afford greater variety of saline material. For the yeast ash, which every one who has tried must have found extremely troublesome to prepare, I substituted the same weight of ashes left after burning a large amount of loppings from various kinds of trees and shrubs; the liquid obtained by lixiviation being filtered, and a quantity used in proportion to the estimated weight of dissolved solids. It seemed to me that the salts obtained in this way would be more likely to afford suitable pabulum for the growth of different organisms than those derived from one particular species of fungus. Thus, my Pasteur's solution had the following composition:—

Water from the tap . .	5000 grains.
Lump sugar	250 „
Tartrate of ammonia . .	50 „
Salts from wood ashes . .	5 „

It happened that the alkalinity of the ashes exactly counterbalanced the acidity of my specimen of tartrate of ammonia,

so that I had a perfectly neutral solution to work with. The flask was prepared and the fluid introduced as above described for milk, but the boiling was done by the direct flame and was continued only ten minutes.

The turnip infusion was prepared by boiling peeled white turnips, in about enough water to cover them, till they were soft, reducing each to a mash with a little additional water, filtering, and keeping the filtrate at 212° F. for half-an-hour, as in the case of the milk.

The "artificial milk" required special preparation. A solution of 160 grains of milk sugar in ten ounces of tap water, which is about the proportion in milk according to 'Miller's Chemistry,' was subjected to the temperature of 212° F. for an hour and a quarter in a flask prepared and arranged as for the milk. Next day, the fluid being of course cold, I added five drachms of the white of a raw egg, the shell of which had been treated twelve days before with one to twenty carbolic acid solution for an hour and twenty minutes and then wrapped in carbolized cotton, a process which, I may remark, preserves eggs from putrefaction, apparently for an unlimited period, although the carbolic acid leaves the cotton in a few days, and that which was applied to the egg shell does not penetrate sufficiently to produce any coagulation whatever of the albumen; and I have lately eaten an egg which had been prepared in this manner more than three months before, and for the last fortnight had been kept at 100° F. A large pipette having been purified by heat, and protected from the entrance of dust in cooling by means of carbolized cotton, a plug of which in the upper end served the further purpose of preventing the entrance of organisms into it from a syringe with which it was connected by means of a caoutchouc adapter, a small hole was made in the shell of the egg with carbolized fingers and heated knife, and the narrow end of the pipette being inserted between the yolk and the shell, and a piece of carbolized cotton wrapped round the pipette so as to cover the orifice and exclude dust, almost all the white was extracted without interfering with the yolk, and transferred at once to the sugar of milk solution in the flask, the cotton round the pipette serving as a temporary screen, for which a substantial cap of the same material was substituted on removal of the pipette. Twenty-four hours later, the flask having in the interval been occasionally agitated to diffuse the albumen, a syphon was introduced with the peculiarity that a piece of sponge was tied over the end of the shorter leg to serve as a filter for excluding the shreddy undissolved residue of the albumen,

the sponge being of course purified by the boiling. The artificial milk was thus obtained with only trifling turbidity when decanted into experimental glasses, and the stock in the flask has remained unchanged to the present time (Sept. 1873) more than three months after it was prepared.

The urine was not boiled at all, but was obtained altogether unaltered by a very simple process, depending upon what appears to be a fact of high interest both physiologically and pathologically, that a mucous canal in a state of health does not permit the growth of foreign organisms in its immediate vicinity, so that preliminary external application of a carbolic lotion (1 to 40) is sufficient to ensure an uncontaminated state of the fluid, which, with its unaltered mucus, is a much more favorable nidus for organic development than after boiling.

One other piece of apparatus requires a short notice, viz. that used for withdrawing fluid from the experimental glasses for inoculation or examination. The most convenient means for this purpose I have found to be what may be called a "syringe pipette," consisting of a small syringe with a piece of glass tube connected with it by a caoutchouc adapter, the junction being self-supporting but yielding (as distinguished from rigid). This last property permits the use of a very delicate tube without risk of breakage when it touches the side of a glass; and it is of great importance that the tube should be of as *thin* glass as possible. It can then be heated fully when dry by once drawing it quickly through the flame of a Bunsen's burner, and a few seconds suffice for its cooling. The tube, which is about a line in diameter, is drawn out a little at the end, and is bent at an obtuse angle about two inches from the syringe; so that the latter is not held over the liquid during the process. Care is taken not to drive any air from the syringe into the tube after heating the latter, and rather more of the liquid than would suffice for inoculation is taken up, so that the part left in the tube may protect that which is ejected from air from the syringe.

To the general reader these details may seem almost unpardonably minute, but for any one who is desirous to repeat similar experiments I venture to hope they will not be found so.

On the 14th June I drew off for the first time some milk from the flask which was exhibited to the Royal Society of Edinburgh in April as having its contents still fluid, and therefore probably unaltered, though prepared seven weeks previously, and under difficulties as compared with the material of later experiments, inasmuch as it was boiled by the direct flame of the lamp, the extreme inconvenience

occasioned by the frothing of this flask having led to the suggestion of the boiling water bath above described. Also the cotton wool over the mouth was not carbolized, a piece of muslin between the cotton and the flask being alone treated with the ethereal solution of the acid. Nevertheless, the cotton filter had proved efficient in spite of the often repeated rapid rushing of air into the flask which must, of course, have occurred whenever the lamp was removed to prevent the froth from reaching the cotton. For the milk when decanted just four months after the boiling, proved perfectly good, having a slight flavour of turnip as might be expected of winter milk; its reaction showed the peculiar character now known to be possessed by that fluid when fresh, purpling blue as well as red litmus paper, and the microscope showed no appearance of organisms or of the granular masses of deposited casein often seen as an early indication of fermentative change, while the milk globules were bright and unaltered.

These observations were made upon the first two or three drachms that flowed from the syphon, received into an unprepared glass, as should always be done to wash out any residual water from the tube, and thus ensure uniformity of the contents of the experimental glasses. Of the latter, one was at once exposed in my study by removing the shade and glass cap to receive any organisms that might fall into it, and was covered again with cap and shade after fourteen hours, including the night and early morning in which the furniture was "dusted" with a cloth by the servant, but the glass carefully avoided. It was then placed beside the other glasses in a cupboard, the temperature of which varied from about 65° to 70° F.

On the 20th of the month I observed for the first time a delicate filamentous fungus on one part of the side of the glass, extending upwards from the milk for about an eighth of an inch; and at the same time a semitransparent layer which had been noticed for about two days previously at the surface of the milk was found to have increased in thickness. Two days later this layer had attained a depth of $\frac{1}{6}$ inch, and I proceeded to investigate its nature, thinking it probable that it might be a change induced by the growth of the fungus. But on trying to take up a portion with the syringe pipette, I encountered a most unexpected difficulty in extreme viscidity of the liquid. I had before observed the effects produced upon milk by thirteen different organisms, including six distinct kinds of bacteria, but though the products had differed extraordinarily

in colour, reaction and consistence, viscidity had in no case been witnessed. Here, however, the upper part of the milk had been converted into the most viscid substance I ever saw. When I at length succeeded in extracting the pipette without any of its contents getting upon the outside of the glass, I found that on touching any object with the delicate end of the tube and withdrawing it, the tiny drop became extended into a thread a foot and a quarter in length, as delicate as the finest spider's web and barely visible from its tenuity. I afterwards amused myself with spinning webs from one object to another. When dry they exhibited considerable tenacity, and thicker ones broke with an audible snap when subjected to longitudinal traction, while the finer ones floated like gossamer in the air. Here, then, was an amazing chemical change effected in the milk, and one of great interest with reference to the elaboration of mucus and other viscid secretions in the animal economy. On applying the microscope I found no fungus filaments, but multitudes of motionless bacteria, such as are represented in Pl. XIX *m*, very minute and delicate, and often showing a peculiarity only badly represented in the specimens drawn, viz. that of having one part of the organism of much higher refractive power than the rest. In the lower part of the glass similar bacteria were seen in active movement, often curiously wriggling and sometimes rotating completely round a transverse axis. The reaction of the milk was also changed, distinctly reddening blue litmus paper and not affecting red.

Next day I introduced into another of the glasses of milk a morsel of the viscid substance by means of a pair of mounted needles passed through the flame. A glass of the artificial milk above described, which had been decanted for seventeen days and had undergone no change, and a glass from a flask of Pasteur's solution which had been prepared on the 11th of February and remained brilliantly clear, were also similarly inoculated.

In the course of two days observing a translucent layer, about a line in thickness, at the top of the milk in the second glass, I removed some for examination. It was distinctly acid in reaction but uncoagulated, and when a drop was diffused on a glass plate the liquid was seen to be generally thin and turbid, but studded with transparent specks which, when touched with the point of a needle, could be drawn out into threads like the viscid material of the first glass On applying the microscope to one of the transparent specks, multitudes of motionless bacteria were

seen, such as are represented at *o*, Pl. XIX, shewing in a striking manner the peculiarity before described, of having their extremities of different refractive power from the rest. The thin turbid part, on the other hand, was a finely granular fluid in which similar bacteria were seen in much smaller numbers, some of them moving freely, while others were motionless, the latter being each surrounded with a transparent halo of greater or less extent as is shewn at *p* and *q*, Pl. XIX, and in some cases, the transparent areas surrounding different bacteria were confluent. These were evidently miniatures of the transparent specks visible to the naked eye; and they seem to me beautiful examples of a change effected by bacteria in the surrounding medium, whether due to vital action of the organism or to some substance (a so-called chemical ferment) emitted from it during life or after death.

The moving bacteria, it is to be remarked, had no transparent area around them, nor were they able to penetrate those that surrounded the motionless ones, proving the substantial character of the latter.

The artificial milk and Pasteur's solution were turbid the day after inoculation: and in the former, which I examined microscopically, were seen active bacteria of extreme minuteness, looking like mere pairs of granules, which on the following day had given place to others of larger size and of the same sort of characters as those of the milk, as shown at *n*, Pl. XIX. Similar bacteria were also seen at this time in the Pasteur's solution. But neither then nor at any subsequent period was there any viscidity of the general liquid in either of these glasses, implying that the viscid substance was no essential appendage of the organism, but the result of its fermentative action upon particular materials.

It is, however, to be added that in the course of the next month a deposit occurred upon the sides of both these glasses such as I never saw under any other circumstances, constituting a film which, in the artificial milk, resembled coagulated fibrin in its toughness, and in the Pasteur's solution was tenacious though not viscid, as if the motionless bacteria which constituted the deposit in each case had been glued together by a minute quantity of some intervening substance.

The next observation which I have to record has reference to the origin of bacteria. It will be remembered that a filamentous fungus made its appearance on the interior of the first milk glass six days after its exposure. The growth continued to spread, and by the tenth day, as it had a bloom indicating probable fructification, I scraped off a small

portion from the glass by means of a tenotomy knife washed with strong carbolic solution and dried in the flame, and examined the specimen in a drop of water with the microscope. It proved to be a fungus of great beauty composed of very delicate branching filaments (*a*, Pl. XIX), bearing spores (conidia) often septate, characterised by a raw sienna tint (*c*, Pl. XIX) which was often distinctly seen to be confined to an external envelope, affording, what is unusual with fungi of such minuteness, the means of definite recognition, and of ascertaining with precision the three modes of germination above alluded to (see p. 381). Many of the spores were seen to have produced thick sprouts to form young plants. Of these *d* has been sketched because it happened that, while part of the brown envelope had been consumed in the process of germination, a portion still remained for identification. Other spores were observed in toruloid pullulation, as is seen at *e* in a mass still connected with the parent filament, and at *g* in a free and septate spore, while *f* was either a spore multiplying by pullulation, or a young plant of a brown colour. For here and there young plants were seen like *b* retaining the brown investment of the spores; and hence, as a dark coloured coat of threads and spores is the special character of the order Dematiei among hyphomycetous fungi, and as de Bary has given the name *Dematium pullulans* to a closely allied microscopic fungus,[1] I have ventured to suggest for this species the name *Dematium fuscisporum*. Further, the spores were often seen to give off exquisitely delicate threads as at *i* and *k*, while in *h* we have a combination of this delicate sprouting with toruloid pullulation in the same spore. Finally, there were observed in abundance among the filaments free bodies like *l* exactly resembling in form, size and refractive power portions of these delicate sprouts. Some of them, not sketched, were seen to be branched, and yet, though in this respect and in the absence of the double rod-like character they deviated from the most typical form of bacteria, their bacteric nature was rendered indubitable by characteristic movement observed in several instances. I may add that in *k* that which is sketched as a branch of the delicate sprout was seen to oscillate from the position indicated to that of the dotted line, as if about to detach itself; though this is an observation to which I do not wish to attach much importance, as the same appearance might possibly result from accidental adhesion of a previously free bacterium. Taking the observation as a

[1] See 'Morphologie und Physiologie der Pilze,' &c. Von Dr. A. de Bary, Leipzig, 1866, p. 183.

whole it affords proof positive of three distinct modes of germination of the spores of one and the same fungus, while there seems little reasonable doubt that the third mode was the source of the bacteria.

It will be remarked that the bacterium which grew thus abundantly among the filaments of the *Dematium* on the dry glass differs entirely in appearance from that which was found in the milk and produced (as I think we are justified in saying) the viscous fermentation. And there is reason to think that they were in reality two entirely different species, and that the one derived, as it appears, from the *Dematium*, (or some other exactly like it morphologically), which I have indicated in the plate as *Bacterium No. II*, existed in the milk along with that of the viscous fermentation (*Bacterium No. I*), though the latter took the precedence in development, so that the former escaped notice in the first instance; as so commonly happens when germs of different kinds are introduced together into the same medium. For having inoculated a glass of fresh urine on the 30th July with a portion of the viscid material from the second milk glass, the product which first showed itself five days later by dimness of the liquid had none of the characters of *Bacterium No. I*, but resembled in elongated and curved form as well as in dimensions the one derived from the *Dematium*, see Pl. XIX, *Urine, 4th August*. It was of course conceivable that the appearances in question might be merely the result of a modification of *Bacterium No. I* by the new medium in which it grew; the other alternative being that two bacteria had existed together in the milk, but that *Bacterium No. I* was either incapable of growing in urine or had lost its vitality during the five weeks which had elapsed since its introduction, while *Bacterium No. II* had survived. The last appears to have been the fact; for on inoculating milk and Pasteur's solution with the new Bacterium, while it throve in both it retained the characters that it had in the urine and occasioned no viscidity of the milk. And further, when introduced into artificial milk, in which *Bacterium No. I.* grew so rapidly, *Bacterium No. II* failed to grow at all, the fluid remaining unchanged for the twenty-six days during which it was kept under observation.

Some other points were observed regarding *Bacterium No. II* which appear of sufficient interest to be placed on record. When first seen in the urine it was unbranched, and exhibited rotatory movements; but when again observed two days later it was found of larger size, and often distinctly branched, see Pl. XIX, *Urine, 6th August*, and entirely destitute

of motion. On this day a minute drop of the urine containing the organism in this condition was introduced into a glass of turnip infusion decanted from a stock of that liquid which was prepared on the 24th of February, and had then furnished the supply for twelve experimental glasses, but which retained its original characters as regards aspect, fresh odour, and faintly acid reaction, while the microscope revealed no organisms. After two days bacteria made their appearance of the characters shown in Pl. XIX, *8th August*, resembling those first seen in the urine in being unbranched, and even more active than they, with wriggling onward movement. Two days later the bacteria were again motionless and of larger size, and often manifestly branched, see Pl. XIX, *10th August*, the turnip infusion having now acquired a smell like that of strong turnip soup. Again four days later, the glass shade having lost all smell, I supposed the fermentation to be over; but on examining a drop I was surprised to find that bacteria were present in abundance, but that all the large and branched ones had disappeared, and in their place was a progeny more minute than any seen before, showing sometimes the double rod form most characteristic of bacteria, see Pl. XIX, *14th August*, and exhibiting active movements of rotation and wriggling. The only explanation that suggested itself to my mind was that some material of limited amount in the turnip infusion yielded under the fermenting influence of the bacteria a volatile product (the same, perhaps, that caused the soupy smell) which, while it remained, exercised a modifying influence upon the organism, resulting in the branched and motionless variety, but on escaping, as indicated by the odourless state of the fluid, left the bacteria to return to their former shape and active movements. And this view was confirmed by the result of inoculating a second glass of the turnip infusion from the first on the 14th August, when the bacterium had the minute and active state for the second time. For precisely the same series of changes of the organism was then repeated, as is sufficiently shown by the sketches, Pl. XIX, *August 15th, 18th, and 20th*. I dwell upon these circumstances because they afford an example of modification of bacteria under different conditions of the same medium, and also an instance of branching, which has been spoken of by Cohn in his recent work as something altogether foreign to this class of organisms.[1] I also venture to hope that facts like these will tend to give the reader additional confidence in the trustworthiness of the mode of investigation.

[1] Op. cit., p. 139.

One other circumstance with regard to *Bacterium No. II* seems deserving of mention. As already stated, when introduced into a glass of boiled milk, it grew rapidly, having after three days the appearances shown in Pl. XIX, *Milk, 18th August*, with active movement. There was, however, up to this time no change in the aspect, odour or reaction of the milk. But in the course of a few days the upper part of the liquid assumed a peculiar golden yellow tint, and a fortnight after inoculation the appearance was almost as if the yolk of a bantam's egg were floating on the surface, while there was also some similar yellow material deposited at the bottom of the glass, and the main body of the milk had assumed a cream colour. The reaction was now distinctly though not strongly acid, but the glass shade had no sour smell, a very faintly urinous odour being the only one perceptible. The main body of the milk was a very soft coagulum, but the upper part was a thin transparent liquid, the bright yellow material being deposited at the junction of the two. On examining a portion of the yellow substance with the microscope, I could discover nothing but a mass of motionless but unbranched bacteria such as are shown in Pl. XIX, *1st September*, and I could only conclude that the bacteria were themselves of yellow tint though too minute to show it under the microscope. Yet it is a curious circumstance that the same bacterium in Pasteur's solution had not this colour, but produced a pale pink tint by the deposit which it formed at the bottom of the glass. At this period I was obliged to suspend my observations, but from what had been seen in the last few days it appeared that the bacteria were converting the coagulum into a transparent liquid, for the upper translucent layer was daily increasing in thickness. On looking at this time at the second milk glass, in which the viscous fermentation had occurred at an earlier period, I found that the viscid upper part had changed to a similar golden yellow colour, and under the microscope I found that *Bacterium No. I* had disappeared, and given place to *Bacterium No. II.* This yellow colour in milk I never saw caused by any other organism.

The last observations which I have now to relate refer to the commonest of all the fermentative changes to which milk is liable, that which results in the rapid evolution of lactic acid, and consequent precipitation of the casein in the form of curd, a change which was attributed by Pasteur, so early as 1857 to the operation of a special organism.[1] The

[1] "Mémoire sur la Fermentation appellée Lactique," 'Annales de Chimie et de Physique,' 3me série, tome lii, 1858.

frequency of this change in milk does not, however, appear to depend on specially extensive dissemination of the ferment, but rather upon the circumstance that the organism which we are about to study, when it does gain access to milk, takes the precedence of others in development, and that dairies being places in which this particular ferment abounds, the milk supplied from them is sure to contain it, as they are at present managed. For it is a remarkable fact, and one well worthy of the consideration of the dairyman, that while milk supplied for domestic use will turn sour in summer weather within twenty-four hours, yet of all the many instances in which I have observed alterations in milk caused by organisms introduced through atmospheric exposure, in no single case did the true lactic acid fermentation occur. Some organisms have given rise to a primary alkaline alteration, strong or feeble, some have been neutral in their effects, while others have produced an acid condition indeed, but only feeble and slowly developed.

It seemed worth while before closing this investigation, in which fermentative changes in milk had occupied a prominent position, to apply our method of inquiry to the most frequent and therefore the most interesting of them all. Accordingly on the 14th of last month, August, I obtained from a dairy near Edinburgh, pervaded with the usual sour smell, about a pint of milk said to have been taken from the cow four hours previously and tasting perfectly fresh, the dairy woman bailing it out with a tin vessel from the pan in which it stood into a clean glass bottle which I had provided. One hour later about ten ounces were introduced into a flask purified by heat, and were subjected to the temperature of 212° F. for three quarters of an hour, the arrangements being such as have been fully described above, see p. 5, and on the following day four experimental glasses were charged each with about half an ounce of the milk by means of a permanent syphon (see above). The first milk that came from the syphon, received into another glass, had the taste of perfectly fresh boiled milk, it purpled both blue and red litmus paper, and exhibited under the microscope nothing but milk globules of all sizes including extreme minuteness. Meanwhile, the milk remaining in the bottle had undergone the usual change. At noon, twenty-three hours after it was taken from the cow, it tasted distinctly sour though still fluid, and sharply reddened blue litmus, and on microscopic examination motionless bacteria were seen in considerable numbers, of soft or delicate character, in pairs, fours, and chains (*Leptothrix* filaments) as repre-

sented at *a* in Pl. XX. The milk examined was in a wine glass into which it had been poured from the bottle, and this was kept covered till 5 p.m. when a small drop was taken out for inoculation of one of the glasses which we may term *Boiled Milk I.* It was now more sour to the taste, and more sharply acid to litmus, and when diffused between plates of glass exhibited small white masses which the microscope showed to be granular (deposited casein) while the motionless bacteria before observed were again seen in abundance. The glass also contained some larger portions of soft curd. Next day at 8.30 a.m., or fifteen and a half hours after inoculation, *Boiled Milk I.*, though unaltered in appearance, had communicated a faintly sour smell to the air under the glass shade, while the smell of boiled milk was gone. A drop removed by pipette reddened litmus more than on the previous day, though still faintly blueing red paper, and under the microscope motionless bacteria were seen in considerable numbers exactly similar to those observed in the unboiled milk, except that there was greater variety in their size, some being considerably larger, as shown in the plate at *b*. At 5 p.m., twenty-four hours after inoculation, the glass shade gave a pleasant smell of slightly sour milk, and the reaction was sharply acid, but the milk was still fluid, and next morning rather more than thirty hours after inoculation the milk had set into a solid mass.

On the same day (15th Aug.) that Boiled Milk I was inoculated as above mentioned, parallel experiments were made with turnip infusion and with urine, each of which received a minute drop from the same glass of sour milk. The turnip infusion was from the stock prepared in February, having both naked-eye and microscopic appearances unchanged; and the urine was a glass prepared at the same time as that used for *Bacterium No. II*, retaining unimpaired in every respect the characters which it then had, seventeen days before. Neither of these glasses showed any signs of bacteric development on the 16th, the day after inoculation, but on the following day both were manifestly nebulous, and both exhibited under the microscope numerous motionless bacteria. There was, however, a remarkable difference between the organisms in these two glasses. In the turnip infusion the bacteria did not differ very greatly from those in the boiled milk, except that the leptothrix form was very seldom seen, and that the segments of the pairs were sometimes of greater length, while unjointed specimens, also pretty long, made their appearance, as at *c*. In the urine on the other hand the deviations

from the form in the milk were most remarkable, as will be sufficiently evident from an inspection of the plate under *Urine I.* Some indeed, like *d*, were not very different from the original leptothrix form, but even such specimens often exhibited, as that one does, an elongated state of some of the segments of the chain, thus forming connecting links between the leptothrix and the widely different spirillum-like specimens such as *e*. Next day the same sort of appearances were again seen, and an observation made on the previous day was confirmed, viz. that vacuoles were present in the thicker specimens. This is well shown in the sketches *g* and *h*, in all of which there is also a further deviation from the type which has been lately held to be invariable in the entire group of bacteric organisms, and from whence the name schizomycetous, as applicable to a totally distinct order of fungi, has been derived, that is to say these bacteria, instead of multiplying by transverse fission, are plainly increasing by pullulation, that is to say, by shooting out buds after the fashion of the yeast plant; and it will be observed that these sprouts are by no means always in a line with the long axis of the organism from which they spring. Yet that they really were the same bacterium was evident, not merely from transitional forms, but from specimens such as *f*, in which in one and the same chain we have the leptothrix character combined with the long and thick vacuoled and pullulating organism. Similar observations were made on the following day; and now even the smallest and most bacteriform specimens sometimes exhibited a minute vacuole, as is shewn at *i*. These appearances did not startle me as much as they would have done had I not seen something almost exactly similar in an earlier part of the investigation, though in another species of bacterium under totally different circumstances.

Thinking it worth while to try how this organism would behave if transferred from the urine to Pasteur's solution, I used for that purpose some of the old February stock, still perfectly bright, inoculating on the 18th. Next day the fluid was distinctly nebulous as examined before a candle, and under the microscope I found motionless bacteria, not numerous, but obviously of new formation from the delicacy of their aspect, represented at *k*, in Pl. XIX, where they are seen to be of considerable thickness and length of the segments, which present a curious alternation of lightness and darkness in their substance. Though a pair and three are given in the sketch as well as a single one, solitary individuals were much the most frequent. Such was the appearance twelve hours after

inoculation, but when twelve more hours had expired a very great change had taken place. Not only were the bacteria much more numerous, but very much smaller; and instead of being commonly single, were invariably double, having in fact the ordinary appearance of minute bacteria (see Pl. XX, *l*), and to complete the metamorphosis some of these bacteria were seen swimming actively in ordinary bacteric fashion. Two days later the liquid was considerably increased in opacity and I was struck with what I had never seen before in Pasteur's solution, a sort of dirty or dingy appearance, as if a very small quantity of ink had been mingled with the liquid, and the deposit at the bottom of the glass, which was white on the previous day, had now the same dingy cast. Under the microscope the bacteria appeared much as on the last occasion, except that some were even more minute than any then were, so that it was impossible to say, except by their movements, that they were anything more than mere granules (see *m*, in Pl. XX). At the same time active movement was more frequent than before.

I now thought it well to ascertain whether these minute and active bacteria would reproduce in urine the same sort of organism as that which we could not but believe to have been their parents in that fluid. On this occasion, having no more of the fresh urine, I adapted a syphon to a flask which was prepared on the 1st of March, and had furnished the material for numerous experiments, yet retained its original brilliancy as well as odour unaltered, was distinctly acid to litmus and displayed no organisms under the microscope. Twelve hours after the inoculation on the 21st the liquid was already manifestly nebulous, and on examination with the microscope bacteria were found, four or five in every field, differing from those that had been introduced in being very rarely double but long and large and often curved *(vide* Pl. XXI, *a*,) having thus returned to a considerable extent to the condition before seen in urine, but now differing from their former state in that fluid in frequently exhibiting characteristic though languid movements. After twelve hours more the previous condition in urine was still more closely approximated by greater length in the segments, as illustrated by *b*, sketched because it happened to be at rest, though by no means having the longest unbroken segments that were observed. I now inoculated from this glass of urine another (*Urine III.*) that had been decanted on the same day and had remained till then unchanged; and twelve hours afterwards I sketched from this second glass the magnificent example of unjointed spirilliform organism

represented at *h*. At the same time languid movement was seen in many specimens.

To complete the history of the behaviour of this organism in urine it may be added that, after the lapse of another fortnight, the bacteria in this glass were found again motionless and comparatively small, scarcely differing in appearance from those originally seen in the sour milk *(vide* foot of Pl. XXI).

With the view of determining precisely the identity of the minute organism in the Pasteur's solution with the large one in urine, I stocked as follows, on the 21st August, a "glass garden" consisting of a massive piece of plate glass excavated by the lapidary into a broad and deep ditch around a central island, the ditch to serve as a reservoir of air. This glass, together with a thin covering glass, had been exposed to a high temperature between metallic plates to diffuse the heat and avoid cracking, and cooled without access of dust. With heated forceps the covering glass was raised and, a minute drop of the Pasteur's solution with its organism having been mingled with a large drop of urine on a glass plate purified by heat, a little of the mixture was placed on the island. The covering glass was then luted down with melted paraffin, applied, with a hot steel pen, after a drop of water, boiled and cooled under the protection of carbolized cotton, had been placed in the ditch with the pipette to ensure a moist atmosphere. Immediately after this had been done I examined with the microscope and saw the minute bacteria of the Pasteur's solution as shewn at *c*, in the Plate, in active movement. On looking again five hours later I found those bacteria replaced by large ones as seen at *d*, still moving though the movement was now languid. Within this short time the one variety of the organism had been converted into the other. Even if we supposed that the thick ones were of a different kind and that one of them had been present originally in the garden unobserved by me, their large numbers at the end of five hours and the vanishing of the small ones would be equally inexplicable. Hence, I think, we may regard it as demonstrated that the minute bacteria of the Pasteur's solution and the coarse ones of the urine were one and the same organism.

Other more remarkable facts, however, remain to be recorded. On the morning of the 22nd August, wishing to ascertain whether this organism, after being so strangely modified in urine, in Pasteur's solution, and then again in urine, retained the property of inducing the lactic acid fermentation in milk, I introduced a minute drop of *Urine*

No. II into a second glass of milk decanted at the same time as the former, and which we may designate *Boiled Milk II.* Nine hours later test paper already indicated a slight degree of acidity, and bacteria were found, five or six in each field, about as thick as those in the urine of inoculation, and also pretty long, generally single, but sometimes double as shewn at *e*, Pl. XXI. On looking at the same slide four hours later I found that other bacteria, much more minute and shewing active progressive or rotatory movements, were also to be seen, and next morning such minute and active ones were alone discernible in another drop taken for examination. The acid reaction was now more marked, and the acidity continued afterwards to increase, till within three days the milk had set into a solid mass.

But along with the lactic acid fermentation another and very different change took place in the milk during the first twenty-four hours. On first looking at the glass on the morning of the 23rd, twenty-one hours after inoculation, I was amazed to see at the bottom of the glass a deposit about a line in apparent thickness as black as pitch, shewing out in a glaring contrast to the white milk. The black material did not appear to undergo any increase in the course of the day or at any subsequent period. But there was a peculiar sickly, almost putrefactive, smell mingled with the sour odour of the air in the glass shade in the course of the next twenty-four hours, though this afterwards passed off, and by the time that curdling was complete a pure smell of sour milk was alone perceptible. On the 26th I turned out the curd to investigate the black substance. I found it adhering firmly to the bottom of the vessel so that it could be completely cleansed of the curd with a camel's hair brush without being detached; and when I picked it out with a knife its lower surface had a brilliant polish corresponding to that of the glass. It constituted a tough scale, between horny and leathery in consistence, and its upper surface presented numerous smooth round depressions with intervening ridges; and it was plain that the pigment had been precipitated in the form of a heavy liquid, the particles of which had coalesced at the bottom of the vessel and afterwards solidified. The intensity of the colour was strikingly brought out by microscopic examination under my highest power, when even parts of extreme tenuity, as at *g*, Pl. XXI, distinctly shewed the sepia tint of the mass. These very thin parts also afforded the opportunity of ascertaining that the substance was perfectly homogeneous and structureless. In other words, the dark substance was not a coloured organism, but a pigment formed from the milk as the result

of the growth of an organism in it. The small amount of the material at my disposal permitted me to ascertain only that it was insoluble in water, spirit of wine, anhydrous ether and a strong solution of caustic potash, both in the cold and boiling states of these fluids, and was also unaffected by cold nitric acid, but was dissolved by boiling nitric acid, to which it communicated a yellow colour. Heated in a glass tube with access of air it burnt without fusion, leaving a white ash.

The question of course presents itself, what was the cause of this remarkable formation of pigment from the milk? That it was induced by an organism introduced into the milk we cannot doubt. But was that organism the same bacterium that in the former glass of boiled milk, as in the original stock of unboiled milk, produced only the lactic acid fermentation, but altered in function while modified in form by its residence in the other media, or was it some other species, some "pigment bacterium," to use Professor Cohn's expression, coexisting with the lactic acid ferment? Before discussing this question I must direct attention again to the glass of Pasteur's solution from which the second urine glass was inoculated. It may be remembered that at the time of that inoculation there was already present a dingy or dirty aspect about that glass such as I had never before seen in Pasteur's solution. Next day this peculiar appearance was considerably increased, and on applying a pocket lens, I discovered a number of minute dark brown specks disseminated over the glass, even close to the level of the liquid where the surface was vertical; each brown point having a tiny brown streak extending downwards from it. I succeeded in picking up one of these brown specks with the attenuated end of the pipette, and on examination found it made up of a mass of motionless bacteria of ordinary form, themselves colourless, but having sepia-coloured particles disseminated among them of the same tint and intensity of colour as the pigment from the milk, very irregular in form and varying in size from mere points, much smaller than the bacteria, to masses considerably larger, as is seen at *n*, Pl. XX, showing that the pigment, though produced under the influence of the bacteria, as seems clearly indicated by its existing specially among the bacteric masses, yet was, as in the milk, a mere amorphous and unorganized product. Thus, we trace back the pigmentary function to the Pasteur's solution, through the urine, although in the latter no pigment whatever was formed. This is in itself a point of interest, as indicating that the formation of pigment is not essential to the organism, but,

just as in the case of the viscid substance produced under the influence of *Bacterium No. I*, occurs only when the medium in which the bacterium is growing is of a nature fitted for furnishing the requisite materials. Further the knowledge that the organism which produced the pigment was present in the Pasteur's solution and in the urine will aid us in considering the question whether that organism was or was not a different one from the lactic acid ferment, and this we may now proceed to discuss.

Supposing it to have been a separate organism, it is not at all likely that it found its way by accident into the first urine glass or the Pasteur's solution during the brief periods of exposure for inoculation or withdrawal of fluid for examination. For in no single instance have I known bacteria introduced before in this way. Nor can it have existed diffused through the original supply of milk, seeing that no pigment was produced in that stock or in the glass *Boiled Milk No. I* inoculated directly from it. We can only imagine it introduced from the original supply by supposing that it had entered the unboiled milk immediately before the inoculation of the first urine glass, and was all taken up in the drop used for the purpose; a contingency possible but not probable.

But even if we admitted that, in spite of the slenderness of the chance of such an occurrence, a separate "pigment bacterium" had made its way accidentally into the first urine glass or that of Pasteur's solution, we should find ourselves confronted by a further series of improbabilities. We should have to suppose that the two bacteria thus coexisting in the two fluids were both modified in form in the same manner by the two media, both becoming coarse and long-segmented in urine, and both minute and of ordinary bacteric aspect in the Pasteur's solution; for none of the minuter kind were seen in the former fluid, nor any of the coarser sort in the latter. Further, we should have to suppose that the "pigment bacterium," when introduced into milk, grew with great activity for twenty-four hours and then suddenly perished. For we have seen that no further deposit of pigment took place after the first night, although the milk remained fluid considerably longer, and on microscopic examination of a drop from the upper part of the glass next day, when granular masses of casein showed that coagulation had begun, I discovered not a vestige of pigment in it. And in further proof that the pigment bacterium, supposing such a separate organism to have been present, had died, I found that a bit of the curd introduced from this glass at the close of the

third day into another glass of the same boiled milk, gave rise to the lactic acid fermentation, pure and simple, with no formation of pigment, and none of the putrid odour that had attended the pigmentary formation in the other glass. It may, perhaps, be suggested that the "pigment bacterium" was poisoned thus early by the lactic acid generated under the influence of the other (supposed) organism. But unfortunately for such a view, we find the same transient character of the pigmentary function in urine as in milk. For, as has been before mentioned, the day after the inoculation of *Boiled Milk No. II* from *Urine II* (resulting in the pigmentary fermentation), I introduced a drop from *Urine II* into another glass of the same urine with the result of reproducing in great beauty the long unjointed form of the bacterium. After two days more I inoculated from this *Urine No. III* a fourth glass of the boiled milk, in the hope of getting back the pigmentary formation. But no such thing occurred, merely the lactic acid fermentation. Now it is scarcely conceivable that the "pigment bacterium" (supposing it present) should have perished so quickly in the urine as well as in the milk. For it is to be remarked that the urine was but little changed by the bacteric development that followed the inoculation, retaining its acidity at the close of the two days, while little effect was produced upon its odour. Besides this it must be borne in mind that, if the supposed "pigment bacterium" was derived from the original stock of sour milk, it had before survived a residence for three days in urine, which was the fluid originally inoculated.

On the other hand, if we admit that there was only one organism present, but modified in function as in form by the different media, the course of events is exactly what we might have anticipated. It was in the Pasteur's solution that the pigmentary function first manifested itself, not indeed during the first thirty-six hours, during which it is distinctly recorded that the deposit in the glass was *white*, but in the course of the next day; and it is natural to suppose that it was in this medium, in which the form became so greatly modified, and at the same time the function of active motion conferred upon the previously motionless organism, that the faculty of pigmentary fermentation was also acquired. Then, just as modifications of form assumed by a bacterium in any one medium are more or less quickly lost when the organism is restored to its previous habitat, so should we expect it to be with altered function, and this bacterium, when transferred from the Pasteur's solution to

either milk or urine, would more or less quickly lose the new fermentative property which it had acquired.

One clear instance of acquisition of a new function by the bacterium is presented by the power of active movement which shewed itself for the first time in the Pasteur's solution; so that if we were to adopt the language of some authors who have attributed a most exaggerated importance to movement as a distinctive character, we should say that the organism was converted in that fluid from a bacteridium to a bacterium. But when restored to urine, the organism moved but languidly and after about two days became again motionless. In milk, on the other hand, the power of motion was more permanently retained, and active movements were observed both in the third and the fourth glass of boiled milk as late as five days after the organism had left Pasteur's solution.

There is another consideration which seems strongly confirmatory of the argument against a distinct "pigment bacterium" as the cause of the black deposit in the milk. If it were true that such an organism existed, which, when introduced along with the lactic acid ferment, would produce this striking effect, black milk would be a thing of frequent occurrence; whereas this is, so far as I am aware, the first time such a thing was ever seen. But if it be asked, why was it that this unheard of appearance showed itself in my experiment? the answer is that the conditions of the experiment were such as to afford the organism opportunities which it had probably never had before. Never before, in all probability, was this organism allowed to develop unmixed with any other in urine and Pasteur's solution consecutively. For while this ferment takes the precedence of others in milk, such is far from being the case in urine, and very probably in Pasteur's solution also. How far the previous residence in urine may have predisposed this bacterium to assume the pigmentary fermentation in Pasteur's solution, further experiment can alone decide. Suffice it to say, meanwhile, that the conditions under which the organism grew were novel, and therefore novel appearances need not surprise us. The case seems exactly parallel to that of *Bacterium No. I.* Never before, perhaps, was milk converted into so viscid a material as it was under the influence of that organism, simply because other organisms which would have interfered with the viscous fermentation were for the first time excluded.

I have dwelt at what will, I fear, be thought tedious length upon this discussion, because the conclusion arrived at seems

to me of extreme importance. For if the same bacterium may, as a result of varied circumstances, produce in one and the same medium fermentative changes differing so widely from each other as the formation of lactic acid and that of black pigment in milk, it becomes readily conceivable that the same organism which under ordinary circumstances may be comparatively harmless, may at other times generate products poisonous to the human economy. We can understand, for instance, a thing that has at an earlier period of my practice as a surgeon often puzzled me, though now, happily, under the antiseptic system of treatment, I never have occasion to witness it, viz. the development of hospital gangrene beneath dressings left for a long time unchanged, whereas in the same hospital ward sores dressed daily continued healthy. Assuming what analogy leads us to suspect, that some organism is the cause of the disease, why should the special virus of hospital gangrene become introduced into a sore under the former condition more than under the latter? We now see that it is not essential to assume the existence of a special virus at all, but that organisms common to all the sores in the ward may, for aught we know, assume specific properties in the discharges long putrefying under the dressings. Similarly, we can imagine the unhealthiness of an old uncleansed hospital as caused not by the introduction into it of new organisms, but by a modification of those common to it and to freshly built institutions. I take these illustrations from surgery; but to the medical reader others of equal importance will readily suggest themselves from physic.

Another peculiarity of the glass of Pasteur's solution remains to be mentioned besides the formation of pigment in it, viz. a *putrid* smell which I never observed before in that fluid, and at the same time, a remarkable taste, a combination of slight bitterness with astringency, the latter so marked as to lead me to test for gallic or tannic acid with a persalt of iron, though without effect.

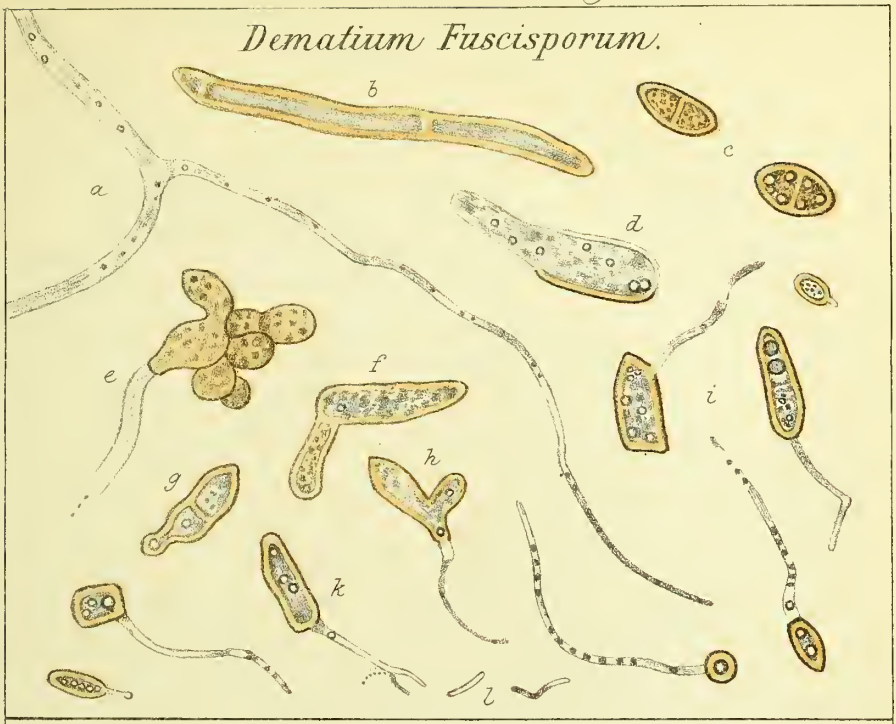
Admitting then that we had here to deal with only one bacterium, it presents such peculiarities both morphologically and physiologically as to justify us, I think, in regarding it as a definite and recognisable species for which I venture to suggest the name *Bacterium lactis*. This I do with diffidence, believing that up to this time no bacterium has been defined by reliable characters. Whether this is the only bacterium that can occasion the lactic acid fermentation, I am not prepared to say; but it seems most unlikely that any other kind will be found combining all the pecu-

liarities of that which we have studied. What fungus it is derived from, if, indeed, it have come from any (for it would be rash to assume that such an origin is universal), I have no means at present of knowing; but, however that may be, it cannot but be right, where we have definite characters of bacteria, to speak of them as species as a matter of convenience, just as is done of various hyphomycetous fungi known to be only inferior varieties of ascomycetous forms.

What are the functions of bacteria with reference to the physiology of fungi, and whether a bacterium derived from a fungus is ever capable of returning to the form of its parent, are questions on which my investigation has thrown no light.

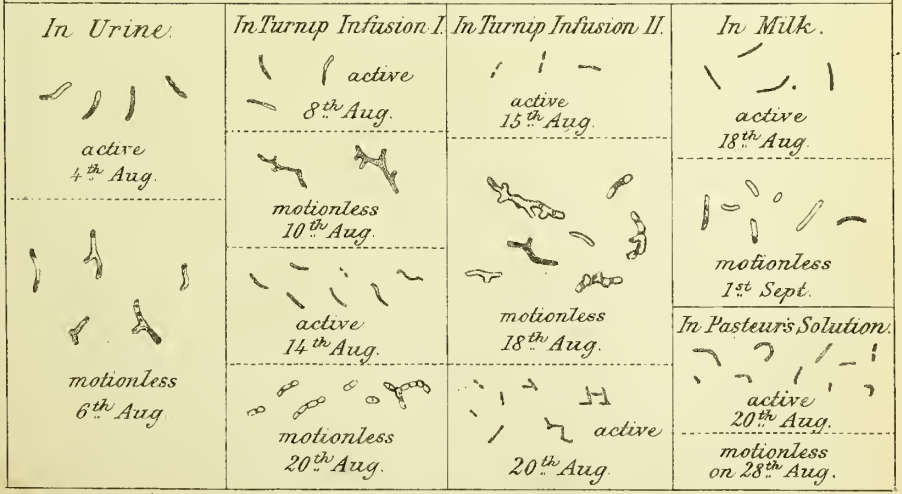
The sketches which furnished the illustrations were all drawn on the scale given at the foot of Plate XXI, either by camera lucida or, in a few cases where the objects were in motion, by eye-piece micrometer, the magnifying power being 1140 diameters. The object-glass which I employed was a tenth immersion lens manufactured by Messrs. R. and J. Beck, the beautiful definition of which was distinctly enhanced by the use of the higher eye-piece.

Dematium Fuscisporum.



Bacterium N.° I.

Bacterium N.° II.



J. Lister ad nat. delt.

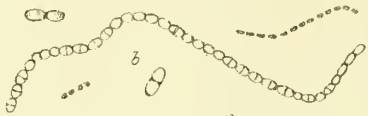
M.c Farlane & Erskine, Lith.rs Edin.r

Bacterium Lactis.

In Sour Milk
used for inoculation

motionless, 15th Aug.

In Boiled Milk I

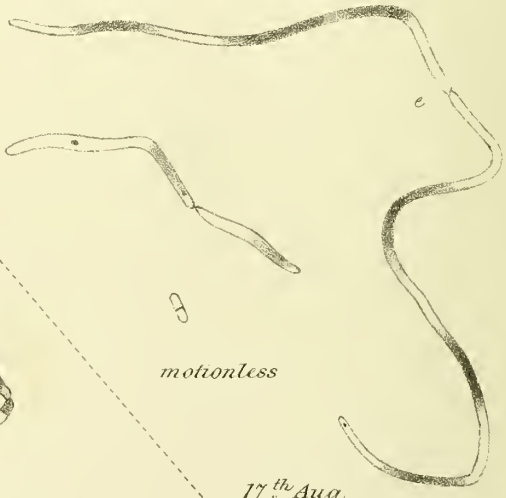


motionless, 16th Aug.

In Turnip Infusion

motionless, 17th Aug.

In Urine I



motionless

17th Aug.

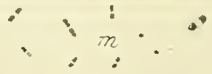
motionless, 18th Aug.

motionless, 19th Aug.

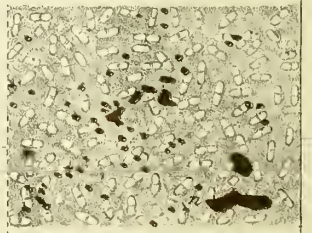
In Pasteur's Solution inoculated from Urine I

motionless
19th Aug 8.15 a.m.

active, 19th Aug. 8 p.m.



very active, 21st Aug.



motionless
25th Aug.

J. Lister, ad nat. del.

Mc Farlane & Erskine, Lith. Edin.

Bacterium Lactis (continued)

In Urine II inoculated from Pasteur's Solution.

languid motion
21st Aug. 12 hours after inocul.n

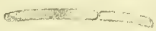
languid motion
22nd Aug. 24 hours after inocul.n

In "Glass Garden" of Urine inoculated from Pasteur's Solution

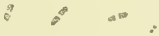
movement active
immediately after inoculation.

movement languid,
five hours after inoculation.

In Boiled Milk II, inoculated from Urine II

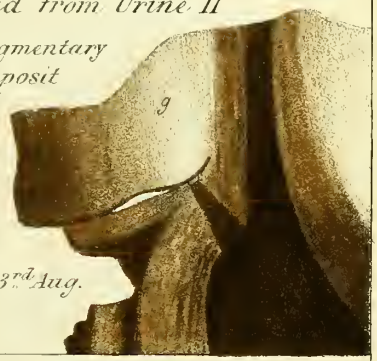


motionless, but smaller ones active.
22nd Aug.



active
23rd Aug.

Pigmentary deposit



of 23rd Aug.

In Urine III, inoculated from Urine II.

languid movement, 24th Aug.

motionless
8th Sept.

one thousandth of an inch.